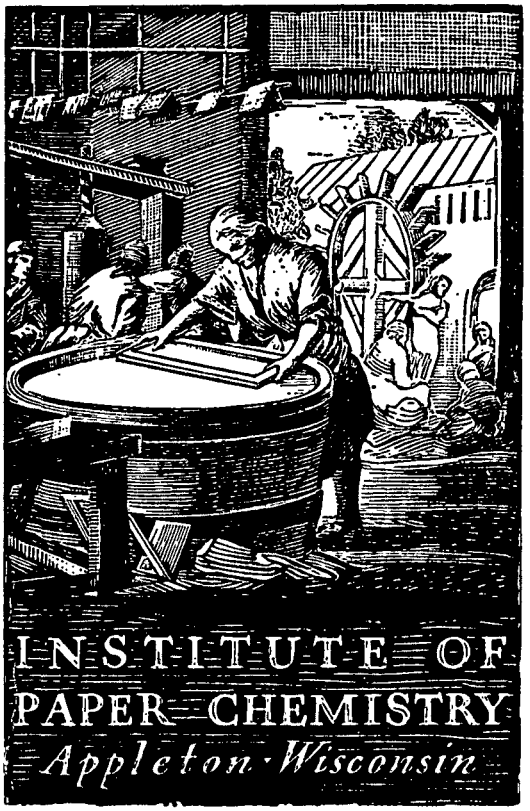

ORGANOLEPTIC ANALYSIS OF FISH TAINTING; THE DEVELOPMENT AND EVALUATION OF A NEW ENVIRONMENTAL ASSESSMENT SERVICE

Projects 3493 and 3508

Report One
A Progress Report
to
MEMBERS OF THE INSTITUTE OF PAPER CHEMISTRY

September 24, 1982

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

ORGANOLEPTIC ANALYSIS OF FISH FLESH TAINTING; THE DEVELOPMENT AND EVALUATION OF A NEW ENVIRONMENTAL ASSESSMENT SERVICE

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by

David F. Sanders

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THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

ORGANOLEPTIC ANALYSIS OF FISH FLESH TAINTING; THE DEVELOPMENT AND EVALUATION OF A NEW ENVIRONMENTAL ASSESSMENT SERVICE

SUMMARY

Segments of the paper industry have expressed a need to determine if treated effluents cause off-flavors or taints in fish flesh. Therefore, Exploratory Projects 3493 and 3508 were defined to develop and evaluate an organoleptic analysis of fish flesh tainting. This test would be offered as an environmental contract service at The Institute of Paper Chemistry. Appropriate food science and current fish tainting literature were reviewed, and versatile data acquisition and analysis procedures for the determination of off-flavor intensity and overall acceptability were chosen for implementation and evaluation.

Three specimen acquisition options, i.e., on-site collection of indigenous fish, in situ receiving stream exposure of caged fish, and exposure of fish to potential tainting materials in laboratory test chambers, were included to meet the anticipated variability in mill specific needs. A versatile sensory evaluation procedure, i.e., an unstructured linear scale scoring test, applicable to evaluation of a series of sample sites or range of effluent or compound exposure concentrations, was selected and designed to eliminate, minimize, or equalize psychological biases inherent in sensory testing.

Initial implementation of sensory analysis procedures, using fish exposed to guaiacol in laboratory test chambers, produced inconclusive results, but identified several procedural problem areas. Subsequent implementation of the sensory analysis procedures (modified according to problem areas identified in the initial test) demonstrated the utility of the procedures and the ability of the panel to detect significant taste differences and produce comparable results in concurrent

evaluations of fish tainting using on-site collection of indigenous fish and in situ receiving stream exposure of caged fish specimen acquisition options at proximate sites.

Subsequent examination of all data sets suggested an additional procedural revision relative to the probable influence of the initial sensory judgment (off-flavor intensity) on the second sensory judgment (acceptability). It is, therefore, recommended that the two parameters be evaluated in temporally separate panel sessions to better ensure independent judgments. The program is now considered a versatile, appropriate, and relatively inexpensive tool for the evaluation of instream tainting effects, or tainting propensities, of whole mill effluents or various process streams.

INTRODUCTION

Overall water quality and indigenous fish populations in many pulp and paper mill effluent receiving streams have improved substantially after widespread implementation of municipal and industrial secondary wastewater treatment. These fishery resources are, in many cases, quantitatively capable of supporting substantial sport and commercial exploitation of "desirable" species. Continued low intensity use of some of these resources may be primarily related to poor eating quality rather than, as prior to secondary treatment, to low densities of these species. Pulp and paper mill effluents, along with other industrial effluents, have been referenced in some literature as causing off-flavors, or taints, in fish flesh. The maintenance of desirability or palatability of fish flesh in effluent receiving streams may be implied under the following goal of the Federal Water Pollution Control Act Amendments of 1972: "water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water."

Regulatory agency concern, relative to the fish flesh tainting issue, has recently been expressed in Wisconsin. Some segments of the paper industry have expressed need for the development of a fish tainting assessment service to determine the degree to which the problem exists. This service would be applicable to mill specific receiving streams where tainting is suspected. The Aquatic Biology Group at The Institute of Paper Chemistry provides a logical framework for this service.

The development and evaluation of a fish flesh tainting analysis program at The Institute of Paper Chemistry were undertaken, and are here presented, as

Projects 3493 and 3508. Overall objectives included (1) a search of the literature for current methods of specimen acquisition and evaluation of tainting in fish flesh, (2) selection of versatile methodologies applicable to anticipated variability in mill-specific requirements or preferences, (3) implementation and evaluation of selected methods, and (4) estimation of contractual costs to mills. All objectives with specific implementation of a single specimen acquisition procedure, i.e., exposure of fish to potential tainting materials in laboratory test chambers, were addressed under Project 3493. Implementation and evaluation of instream effluent exposure specimen acquisition procedures with a slightly revised (according to problem areas identified during Project 3493) procedure for evaluation of the fish flesh were subsequently addressed under Project 3508.

LITERATURE SEARCH

Food science literature was reviewed for background information on proper applications, setup, and conduct of organoleptic tests and analysis of the data. Literature sources concerned specifically with fish tainting were reviewed for background on currently used experimental designs, specimen acquisition procedures, and analysis methods. Major components of a typical fish tainting evaluation program (discussed below) are test specimen acquisition and off-flavor evaluation of the fish flesh.

TEST SPECIMEN ACQUISITION

Three major methods of specimen acquisition have been used historically:

1) On-site collection of indigenous fish
2) In situ receiving stream exposure of caged fish
3) Exposure of specimens to potential tainting materials in laboratory test chambers

1. On-site collection of indigenous fish

Target species are collected from control and experimental sites using appropriate techniques, e.g., electroshocker or nets. Data resultant from this method are indicative of instream effluent exposure. Possible complications include the collection of a sufficient quantity of uniformly sized (or aged) individuals of a particular target species from predetermined sample sites. The major shortcoming of this procedure, however, is the inherent uncertainty of the specimens' previous movement and exposure to tainting materials; this is of particular concern in lotic (flowing water) systems with multiple potentially taint-causing wastewater discharges. The cost of this specimen acquisition option is variable but

can be relatively low when suitable target species are readily available and a minimum amount of time is expended on field collection.

2. In situ receiving stream exposure of caged fish

Test specimens may be purchased from a hatchery or collected from natural sources known not to be contaminated. The fish are then exposed in cages at predetermined control and experimental sites in the receiving stream or body of water (one- to ten-day exposure periods have been cited in the literature as adequate for acquisition of taint; two to four days were most commonly cited). Site-specific exposure to "instream" effluent effects, ease of collection, and stricter control (relative to that inherent in collection of indigenous fish) of extrinsic sources of variation (specimen size, age, origin, and movement during the period of taint acquisition) are the main advantages of this option. Major problems associated with this option include introduced stress on test specimens during collection, transportation, and handling (i.e., maintenance of healthy stock over prolonged exposure periods), weather and geographical complications, vandalism, and higher cost. ASTM (1) recommends this approach and if the above-mentioned problems can be overcome, this option would result in the most reliable site-related data.

3. Exposure of specimens to potential tainting materials in laboratory test chambers

Test specimens may either be purchased from hatchery sources or collected from natural sources known not to be contaminated and then acclimated to dilution water in the laboratory for about ten days. The fish are then exposed to a wide range of compound or effluent concentrations for a period of two to four days according to the procedures outlined by Domtar Research

Centre (2). The range of exposure concentrations is subsequently narrowed, based on results of the wide-range exposure, for determination of the threshold tainting concentration. Loading density, fish stock, age, previous exposure, and physicochemical characteristics of the water can be controlled and replicated. This method is most useful for determining threshold concentrations and for comparing tainting propensities of total mill effluents or various process streams but is less representative of instream effluent exposure conditions.

OFF-FLAVOR EVALUATION OF FISH FLESH

Analytical "Tool"

A wide variety of materials, including hydrocarbons, phenolic compounds, sodium pentachlorophenate, coal-tar wastes, sewage containing phenols, coal-coking wastes, outboard motor exhaust, petroleum refinery wastes, kraft paper mill wastes, wastes from synthetic rubber, explosives manufacturing wastes, algae, resins, and resin acids have been found to cause off-flavor in fish flesh (3). Thomas (3), N.A.S. (4), and Reid (5) list specific compounds and their respective concentrations in water which can cause tainting of fish flesh. These concentrations generally ranged from 0.01 mg/L to 20 mg/L (ppm). The actual concentrations of some compounds in tainted fish flesh (identified as tainted by a human sensory analysis panel) have been found to be less than 0.1 ppb (6), a concentration below the lower detection limits of even GC/MS analytical instruments. Human senses of taste and odor are acute and generally capable of determining the presence and degree of tainting; but they are, in most cases, unable to identify specific compounds. Nevertheless, the use of human sensory analysis panels as the analytical tool in tainting analyses of fish flesh has been widespread (3,6-19).

Factors Influencing Sensory Measurements

Human sensory evaluations are the result of complex sensations involving taste, smell, touch, sight, and hearing, and many outside factors can potentially influence sensory judgment. The value of the human sensory analysis panel tool depends on the objectivity, precision, and reproducibility of the panel response. Extensive efforts must be made to minimize or control variables which can affect human judgment, such as physical conditions of the panel member or testing environment and psychological errors inherent in the procedure.

Larmond (20) recommended test area setup and sample preparation procedures to better ensure independent judgments by panel members and minimize extraneous influences on judgment formation. The testing area should be separate from the sample preparation area, and a slight positive air pressure should be maintained so that odors from surrounding areas will not enter. It should also be quiet, and smoking and cosmetic odors should be avoided. Panel members should be separated, e.g., in individual test booths, and the lighting and color of the room and testing booths should not influence the appearance of the sample. Testing should be conducted during late morning and midafternoon to minimize lingering influences from respective morning and midday meals. Testing on Mondays and Fridays should be avoided because of possible influences from psychological attitudes on these days.

Larmond (20) and Amerine et al. (21) discussed the potential effects that motivation and some psychological errors have on sensory judgments and recommended procedures to minimize or equalize the effects. Human sensory perception is related to motivation, which can be maintained by emphasizing the importance of the activity, reporting results to the panel, and conducting the test in a controlled efficient manner. Expectation, stimulus (and closely associated logical error), and proximity errors, respectively, can be minimized by releasing only minimal information (about

specimens) to panel members before the test, maintaining uniformity in sample appearance, and avoiding multiparameter evaluations on the same sample during a single test session. Contrast error (an exaggerated response elicited when a good sample is tested immediately after a particularly poor sample - or vice versa) and positional bias can be equalized by presentation of samples to each panel member in random order.

Sensory Tests

There are several different categories of taste tests, each with slightly different applicability and requirements. Difference tests are used to detect small taste differences between two samples and require a trained panel. Rank and scoring tests determine how several samples differ in acceptability or on the basis of intensity relative to a specific characteristic or reference specimen. Ranking results in the arrangement of two or more samples in ascending or descending order of intensity or acceptability but disregards the amount or degree of difference between samples. Scoring tests are similar to ranking tests except that samples are scored or rated on a selected scale and an attempt is made to determine a degree of difference. The main disadvantage of scoring tests is the subjectivity of response associated with incremental definition of scores or scale. Descriptive tests are most complex and require a highly trained panel to provide a detailed descriptive evaluation of the sample. Specific tests applicable to each category are discussed in detail by Larmond (20) and Amerine *et al.* (21).

Sample Preparation

Experimental and control specimens should be processed similarly and as soon as possible after collection or completion of *in situ* or laboratory exposure to avoid deterioration of the tissue with associated effects on taste. Although specimen processing specifics varied somewhat between literature sources, fish were

generally killed, decapitated, eviscerated, filleted or not, double wrapped in aluminum foil, labeled, separated by treatments into plastic bags to avoid contamination, and frozen (~ -10°C) (1-3,5-16, 18,19). In general, implemented and recommended processing procedures were quick and consistent within respective studies. Processing for preference-acceptance tests should be typical of that normally used by the consumer.

Cooking of specimens (experimental and control) should be consistent with no alteration or loss of flavor, e.g., no seasoning or cooking oil. Experimental and control specimens should be cooked separately to avoid cross contamination. Oven baking at about 190°C to 230°C for 15 to 30 minutes with no spices or seasoning was used most commonly in the literature reviewed. Samples should be divided into individual portions, placed in coded serving dishes, and kept warm before presentation to panel members in preset (fork, napkin, rinse water, pencil, and ballot appropriate to the test) individual booths.

Data Analysis

Data obtained from sensory tests are generally highly variable, and interpretation, therefore, must generally follow statistical testing. Statistical methods compare results obtained with those expected by chance alone and the results are expressed in degrees of significance (i.e., the probability that the results were caused by chance).

Larmond (20) and Amerine et al. (21) reviewed statistical methods appropriate to various sensory tests. Data obtained from sensory testing of fish are often consistent only for those fish exposed to high concentrations of tainting materials and for the hidden controls, and less consistent for intermediate exposure concentrations (1). These data may not meet distribution assumptions for parametric

analysis of variance tests, thereby necessitating the use of distribution free methods, or nonparametric tests. Nonparametric statistical procedures are described by Sokal and Rohlf (22), and their use is recommended in fish tainting studies by ASTM (1).

METHODS IMPLEMENTATION AND EVALUATION

EXPOSURE OF SPECIMENS TO POTENTIAL TAINTING MATERIALS IN LABORATORY TEST CHAMBERS - PROJECT 3493

Methods for implementation and evaluation were selected on the basis of demonstrated (in the literature) applicability and versatility to best meet (in an unbiased manner) the anticipated variability in mill-specific needs. These methods were designed to yield data on off-flavor intensity and consumer acceptability of fish variably exposed to pulp and paper mill effluents; characterization or identification of tainting compounds in the fish flesh was beyond the scope of the project.

Procedures

Mature yellow perch (60 individuals about 8 to 12 inches total length) were obtained (purchased) from ponds known to have excellent water quality and were kept for about two weeks in the laboratory - 30 fish each in two 722-L aerated tanks filled with dechlorinated water. During this period fungal infection became evident on some fish. The fish were not treated for this infection because of possible effects on taste. Fish which appeared healthy were then selected for laboratory exposure to various concentrations (i.e., 0, 0.01, 0.10, 0.50, 1.00, and 2.00 mg/L) of guaiacol which, according to the literature, bracketed the fish tainting threshold concentration, i.e., 0.082 mg/L (4), of guaiacol in water. Control (10 fish) and experimental (5 fish for each concentration) fish were similarly exposed. One large (> 10 inches) or two smaller (< 10 inches) fish were placed in plastic bags with 20 L of aerated dechlorinated tap water with appropriate amounts of guaiacol. The anticipated exposure period was 96 hours, with 48-hour replacement of test solutions.

Continued occurrence of fungal infection and some mortality was observed, particularly of control fish, after 48 hours exposure. Guaiacol apparently inhibited to some extent fungal growth on experimental fish. To avoid additional stress, exposure was terminated at 72 hours. All remaining live fish were quickly killed, filleted, double wrapped in aluminum foil, labeled, and frozen at -10°C. Although the poor suitability of stressed fish as test specimens was realized, the analysis was carried to completion to identify other procedural problem areas and to gain experience with the procedure.

A 16-member volunteer taste panel comprised of Institute employees and students was organized, informed of the basic nature and purpose of the program, and given preliminary instructions on proper use of the sensory analysis ballot (Fig. 1). Testing facilities, consisting of a large room (relatively free from outside distraction) with portable isolation booths and a separate but proximate fully equipped kitchen with exhaust fans to the outside, were prepared. Sensory evaluation was scheduled for midafternoon (2:00 p.m.) during midweek (Wednesday) about two weeks after specimen exposure. Sample fillets were removed from the freezer one day prior to sensory evaluation, thawed overnight in a refrigerator, the dorso-lateral muscle cut into pieces (~ 1 x 1.5 inches), baked for 20 minutes at 400°F in covered aluminum pans (one treatment per pan) without cooking oil or seasoning, placed in coded ("C" for the identified control; a unique random three-digit code for each treatment including the hidden control) covered glass Petri dishes, and immediately presented (one each "C" and coded dishes) to each panel member for evaluation on a single ballot (a randomly determined evaluation sequence for each panel member).

Numerical data were obtained from the unstructured linear scales by superimposing a seven-part equal interval scale and recording corresponding numeric values.

NAME _____ DATE _____

I have been informed about the nature of the foods to be tasted by this panel

(initial)

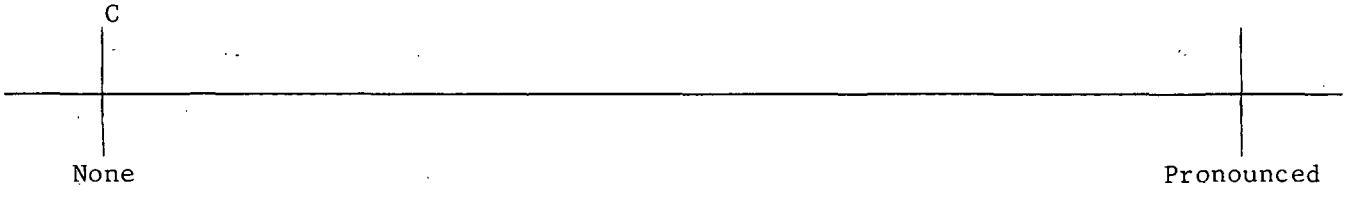
Directions

Taste each sample, in the order indicated below, and make a vertical line across each horizontal line scale at the point that best describes your assessment of the respective property for the sample. LABEL EACH VERTICAL MARK WITH THE CODE NUMBER OF THE SAMPLE.

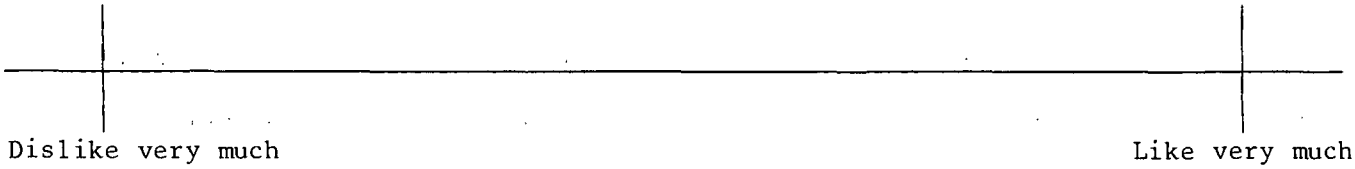
Taste the samples in the following order.

1st	2nd	3rd	4th	5th	6th	7th
C	XXX[a]	XXX	XXX	XXX	XXX	XXX

Property 1. Off-flavor intensity = intensity of any detectable unusual or off-flavor [sample C (control) is recorded for reference]



Property 2. Overall Preference (or Acceptability)



[a]Three-digit code.

Figure 1. Sensory evaluation ballot used for yellow perch exposed to guaiacol in laboratory test chambers.

These data were subsequently tested using statistical analyses appropriate to distributions of the data sets.

Results and Discussion

Although mean off-flavor intensity responses were slightly lower and acceptability responses were slightly higher for fish exposed to sub- and near-taint threshold levels (0, 0.01, and 0.1 mg/L) of guaiacol than for fish exposed to super-taint threshold levels (0.5, 1.0, and 2.0 mg/L), data were variable within treatments and differences were not statistically significant ($\alpha = 0.05$) (Fig. 2, Appendixes I and II). This initial application of the procedure, however, identified several controllable sources of error and variability.

The fish were apparently stressed, by fungal infection, prior to and during exposure to the guaiacol. Stress results in altered metabolic rates which may alter uptake of tainting materials and cause erratic taste panel results (2). This source of error is controllable and would be eliminated by the use of healthy stocks.

Several probable sources of variability were identified after the sensory evaluation session. Panel members indicated that many samples were cool or cold at the time they were evaluated; the practical problems involved in maintaining uniform sample warmth during distribution of a series of small samples to a group of 16 panel members were underestimated during this initial implementation of the procedure. Concern was also expressed by panelists regarding the lack of opportunity to retaste Sample "C" to recalibrate themselves to the off-flavor intensity scale. It was apparent that many treatment evaluations were not being made independently, but rather in comparison with other responses already recorded on the ballot. Adequate amounts of Sample "C" along with suggested recalibration and a separate ballot for each treatment would better ensure independent treatment evaluations.

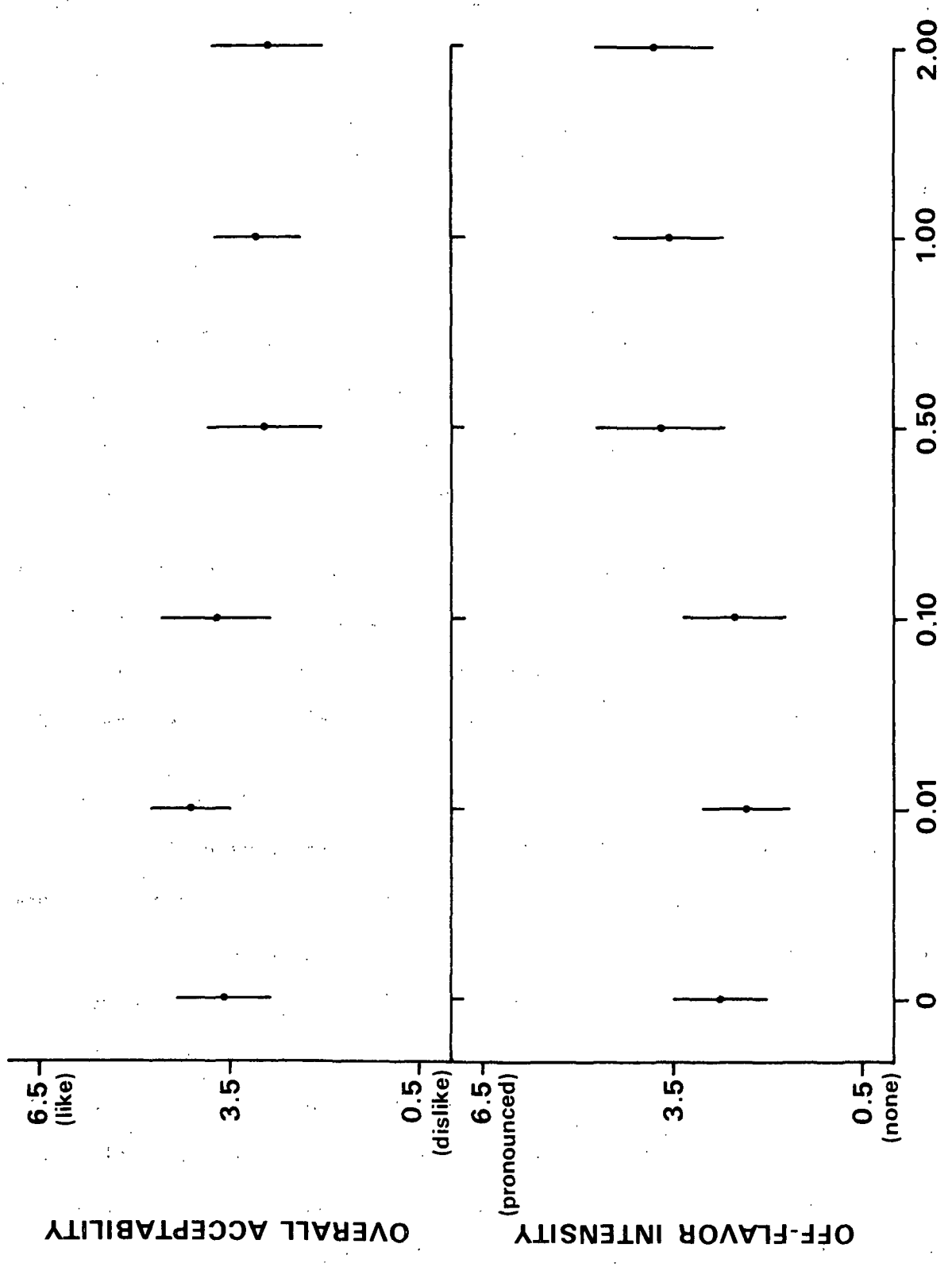


Figure 2. Mean off-flavor intensity and overall acceptability scores, with 95% confidence intervals (vertical lines), for yellow perch exposed to guaiacol in laboratory test chambers

IN SITU RECEIVING STREAM EXPOSURE OF FISH IN CAGES - PROJECT 3508, AND ON-SITE COLLECTION OF INDIGENOUS FISH

Specimen acquisition options for the determination of instream effluent effects (i.e., in situ exposure and collection of indigenous fish) were implemented to obtain specimens for subsequent analyses using a slightly modified (according to problem areas identified during Project 3493) sensory evaluation procedure.

Procedures

These specimen acquisition procedures were concurrently implemented at proximate sites during fall, 1981, in a river system characterized by an upstream reach relatively free from point source pollutional influences and a midstream reach with numerous point source discharges. Numerous dams were also present in the midstream portion of the stream resulting in a series of reaches typically bounded by a dam at the upstream end, followed in downstream progression by lotic (flowing water) habitat, transitional lotic to lentic (lakelike) habitat, and lentic habitat bounded on the downstream end by another dam. A control site (A) was located in the upstream reach of the system; experimental sites (B, C, D, E, and F) were located in the midstream reach in the downstream vicinities of pulp and paper mill effluent treatment facility discharges.

In situ receiving stream exposure of fish in cages was originally attempted using walleye (Stizostedion vitreum), an indigenous and highly sought after game species in the study stream. Walleye (assumed to be of high quality) were electro-shocked from the upstream reach, held overnight in a trap net, transported in aerated holding tanks during early morning hours, and exposed in cages at upstream control and midstream experimental sites. All walleye at all sites, which were apparently in excellent condition when introduced into the cages, died during the subsequent seven-day exposure.

Similar in situ exposure was then conducted using yellow perch purchased privately from a known high quality source. All perch at all sites survived and apparently were in excellent condition after the seven-day exposure. These fish, upon retrieval, were processed and frozen according to previously described methods.

Indigenous walleye were collected (electroshocked) in the downstream vicinities of dams at locations proximate to perch exposure sites. Additional trap netting effort was expended at Site B. Commercially obtained frozen walleye fillets were included for comparison in acceptability testing.

Off-flavor and acceptability of these perch and walleye specimens were similarly evaluated using previously described methods, with the following modifications:

a) After baking and transfer to coded dishes, the samples were organized, by treatment, and maintained on a food warming apparatus, i.e., under infrared lights. Uniformly warm samples were then easily and quickly distributed to the panel.

b) A relatively larger portion of Sample "C" was provided. Reference back or recalibration to Sample "C" was permitted and encouraged throughout the test.

c) Coded samples were evaluated on separate ballots in an order indicated by the sequence of single sample evaluation ballots (Fig. 3) in each booth. (This sequence was randomly predetermined for each panelist to equalize contrast error.)

NAME _____ DATE _____

I have been informed about the nature of the foods to be tasted by this panel

(initial)

Directions

Taste sample XXX and make a vertical line across each horizontal line scale at the point that best describes your assessment of the respective property for the sample.

Property 1. Off-Flavor intensity = intensity of any detectable unusual or off-flavor (sample C [control] is recorded for reference)

Property 2. Overall Preference (or Acceptability)

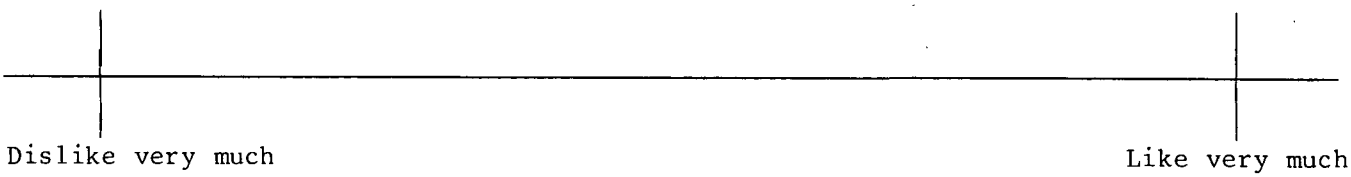


Figure 3. Sensory evaluation ballot used for indigenous walleye and yellow perch exposed in cages.

Results and Discussion

On-site collection of indigenous fish and *in situ* receiving stream exposure of caged fish have commonly been used to evaluate the fish tainting propensity of ambient water quality conditions. Both methods have inherent advantages and disadvantages, some of which influenced the conduct and results of these procedural implementations.

Extensive handling and transportation of fish is a major drawback of the *in situ* exposure specimen acquisition option. A sufficient quantity of uniformly sized walleye were readily collected from the control site, and they were apparently in excellent condition after transportation to exposure sites. All fish, however, including those reintroduced in cages (3 fish/cage) at the control site died during the subsequent seven-day exposure period. Dead walleye observed at the experimental sites were in a more advanced stage of decomposition than those at the control site. This suggests that mortality was related to stress associated with handling and reintroduction into different ambient water quality conditions and possibly (at the control site), also to lack of feeding for the piscivorous (fish-eating) test species during exposure. Walleye were apparently an inappropriate test species for *in situ* exposure.

Yellow perch proved to be a more suitable species for *in situ* exposure. The perch [8.8 to 11.0 inches total length (Table 1)] survived the handling, transportation, and seven-day exposure and were apparently in excellent condition when retrieved from the exposure cages.

Sufficient quantities (for analysis) of indigenous walleye were collected at Sites A, C, D, E, and F (Table II). Only fish from 11.8 to 17.5 inches total length (probably representative of at least two age classes, i.e., III+ and IV+)

were used for organoleptic analysis. If enough fish had been collected, a narrower size range, e.g., 14 to 16 inches total length, would have been preferable. Considerable effort (electroshocking and trap netting) yielded only one walleye (11.9 inches total length, an insufficient quantity for analysis) at Site B and this site was, therefore, deleted from the analysis.

TABLE I

TOTAL LENGTHS OF YELLOW PERCH EXPOSED IN CAGES, FOR SUBSEQUENT ORGANOLEPTIC TESTING, AT SITES A, B, C, E, AND F

Site	Total Length (inches)
A	8.8, 8.9, 8.9, 8.9, 9.1, 9.1, 9.6, 9.7, 9.7, 9.8
B	9.1, 9.4, 9.8, 10.4, 10.6
C	9.3, 9.6, 10.0, 10.6
E	5 fish range ≈ 9.1 to 10.6
F	9.3, 9.8, 10.0, 10.6, 11.0

TABLE II

TOTAL LENGTHS OF WALLEYE TAKEN, FOR ORGANOLEPTIC TESTING, FROM SITES A, B, C, D, E, AND F

Site	Total Length (inches)
A	15.5*, 16.0*, 17.0*, 17.5*
B	11.9
C	12.0*, 15.4*, 17.9, 22.2
D	14.0*, 14.1*, 14.4*, 19.8
E	11.8*, 12.8*, 15.2*
F	14.5*, 15.0*, 15.3*, 16.9

*Fish used in sensory evaluations.

Instream effluent exposure effects on fish tainting as indicated by sensory evaluations of exposed perch were in relative agreement with those indicated by sensory evaluations of indigenous walleye collected from proximate locations. Mean off-flavor intensity was 1.52, 2.06, 4.41, 3.16, and 3.08, and mean overall acceptability was 4.85, 4.23, 2.23, 3.31, and 3.58 for yellow perch exposed at Sites A, B, C, E, and F, respectively (Fig. 4 and 5, Appendix III). Mean off-flavor intensity was 1.96, 3.70, 3.02, 4.09, and 3.03, and mean overall acceptability was 4.34, 2.68, 3.69, 2.37, and 3.46 for indigenous walleye taken at Sites A, C, D, E, and F, respectively (Fig. 4 and 5, Appendix IV). Mean overall acceptability of commercially obtained frozen Canadian walleye fillets was 4.74.

Distributions of the raw data sets were bimodal rather than normal (normality is an assumption of parametric ANOVA testing). The data, therefore, were tested for statistically significant ($\alpha = 0.05$) differences using nonparametric methods, i.e., Kruskal-Wallis test and nonparametric multiple comparisons by simultaneous test procedure. The sensitivity of the Kruskal-Wallis test is at least 0.864 that of parametric ANOVA (23).

Perch exposed at Site C exhibited significantly greater off-flavor intensity and were significantly less acceptable than those exposed at the control Site A and at the upstream-most experimental Site B (Table III, Appendix V). Walleye collected at Sites C and E exhibited significantly greater off-flavor intensity than those collected at control Site A and were significantly less acceptable than commercially obtained Canadian fish (Table III, Appendix VI).

Although results of the two specimen acquisition procedures were generally comparable, some discrepancy was apparent between exposed perch and indigenous walleye results at Site E. Walleye from Site E exhibited subtantially higher off-

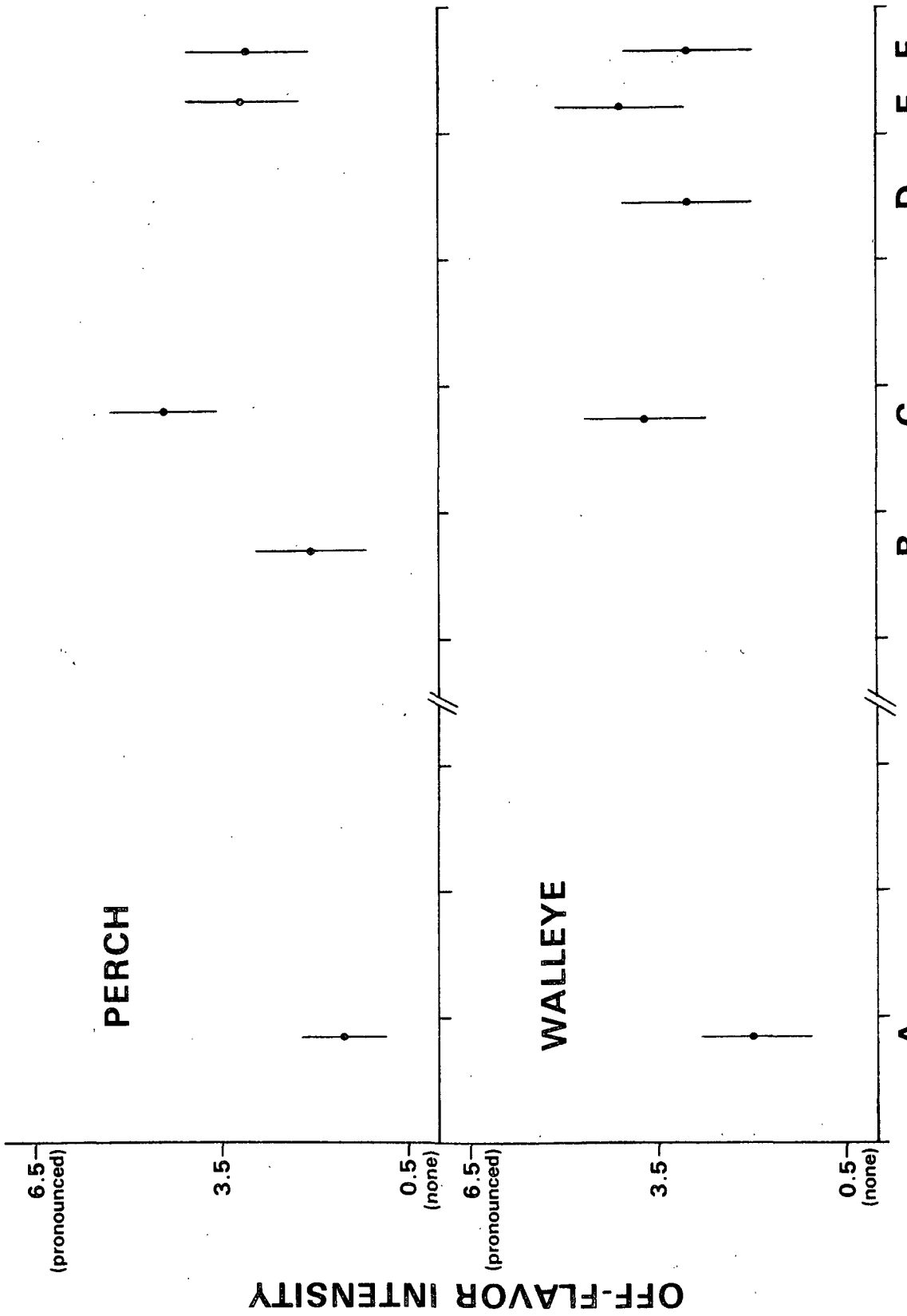


Figure 4. Mean off-flavor intensity scores, with 95% confidence intervals (vertical lines), for yellow perch exposed at Sites A, B, C, E, and F and walleye taken from Sites A, C, D, E, and F

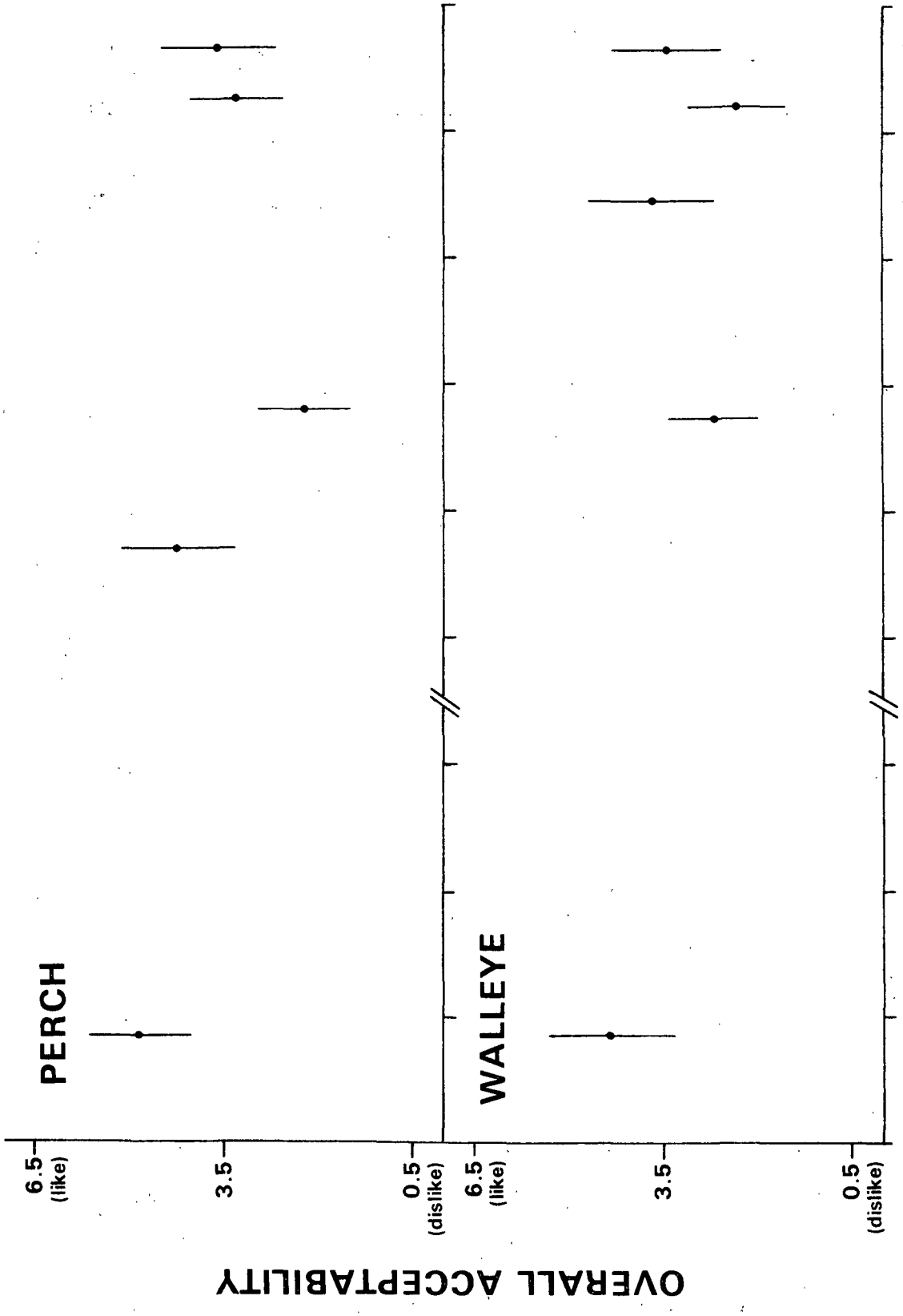


Figure 5. Mean overall acceptability scores, with 95% confidence intervals (vertical lines) for yellow perch exposed at Sites A, B, C, E, and F and walleye taken from Sites A, C, D, E, and F

TABLE III

NONPARAMETRIC SIMULTANEOUS TEST PROCEDURE RESULTS FOR OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY EVALUATIONS OF YELLOW PERCH EXPOSED AT SITES A, B, C, E, AND F, AND WALLEYE TAKEN FROM SITES A, C, D, E, AND F OR PURCHASED, i.e., CANADIAN WALLEYE. (HORIZONTAL LINES CONNECT SITES WITH SIMILAR MEANS; $\alpha = 0.05$)

Off-flavor Intensity

Perch:	A	B	F	E	C
	———	———	———		
			———	———	———
Walleye:	A	D	F	C	E
	———	———			
		———	———	———	———

Overall Acceptability

Perch:	A	B	F	E	C	
	———	———	———			
			———	———	———	
Walleye:	Canadian	A	D	F	E	C
	———	———	———			
		———	———	———	———	———

flavor intensity and lower acceptability than walleye from proximate Sites D and F; this pattern was not observed in the perch exposure data. This discrepancy can be explained through discussion of indigenous fish behavior and physical characteristics of the study area relative to inherent shortcomings of the collection of indigenous fish specimen acquisition procedure.

Walleye in the study stream move upstream during the fall months and thereby concentrate in the downstream vicinities of dams, where they were readily collected during this study. These dams presented essentially impassable barriers to adult fish movement. Walleye collected at Site C could potentially move through about 10 linear miles of stream; walleye collected at Sites D, E, and F could potentially move through about 12, 2.5, and 18 linear miles of stream, respectively. Some indigenous walleye collected at each site were probably distributed, prior to collection, in the deeper downstream lentic areas of the respective stream reaches. The relatively high off-flavor intensity and low acceptability of walleye from Site E (a pattern not indicated in the perch exposure data where extrinsic sources of variability, particularly movement, were rigidly controlled) was a probable result of the structural restriction of these fish, through their life cycle, to the immediate downstream vicinity (~ 2.5 miles) of a wastewater treatment facility discharge.

CONCLUSIONS AND RECOMMENDATIONS

Implementation of the sensory evaluation procedure, modified according to problem areas identified in an initial trial application, identified statistically significant taste differences and produced generally consistent results in concurrent evaluations of site-related tainting using collection of indigenous fish and *in situ* exposure specimen acquisition methods. Observed differences in the results

obtained from the two specimen acquisition methods were probably related to inherent shortcomings of the methods rather than to inconsistent panel evaluations.

Indigenous fish data must necessarily be interpreted with respect to probable sources of variability, e.g., uncertainty about the specimens' previous movement and exposure to tainting materials, and specimen age or size differences between sites. Because of these potential sources of variability, the collection of indigenous fish specimen acquisition method is most reliable for initial or general identification of fish tainting in a body of water.

Site-specific tainting effects are most reliably investigated by in situ exposure of caged fish, where the above-mentioned extrinsic sources of variability are rigidly controlled. Major drawbacks to this method are the possibility of vandalism and the maintenance of healthy stock through collection, transportation, and exposure. Walleye, as determined in this study, is not a suitable test species for in situ exposure; yellow perch, apparently, is a suitable test species.

Specimens obtained from laboratory exposure to tainting materials (during the initial test implementation) were probably not suitable for analysis. It is, however, anticipated that healthy fish stocks could be maintained through laboratory acclimation and exposure, and the method is considered a valid and implementable program option.

Off-flavor intensity and overall acceptability data sets obtained from respective procedural implementations suggested some influence of the initial sensory judgment (off-flavor intensity) on the second sensory judgment (acceptability). This psychological error is referred to as halo effect (20) or proximity error (21). Off-flavor intensity and acceptability, therefore, should be evaluated separately in temporally different panel sessions to better ensure independent sensory judgments

of the two parameters. This and previously mentioned modifications are incorporated into the final procedural outline in Appendix VII. This methodology (specimen acquisition options, sensory evaluation, and statistical analysis of the data) appears to be a versatile, appropriate, and practical tool for the unbiased determination of tainting in fish variably exposed to effluents.

CONTRACTUAL COST

Contractual cost of the procedure would necessarily vary with mill specific needs and location. The following estimates are based on a typical assessment at one mill site or for one effluent. Estimates are based on 1982 billing rates for existing staff.

SPECIMEN ACQUISITION AND EXPOSURE OPTIONS

On-site collection of indigenous fish - collection at six sites	~ $1300 + travel and miscellaneous
In situ receiving stream exposure of caged fish - seven day exposure of purchased fish at six sites	~ $2000 + travel and miscellaneous
Exposure of fish to potentially tainting materials in laboratory test chambers, four-day exposure of purchased fish to six concentrations of effluent or compounds	~ $2000
SENSORY ANALYSIS, DATA ANALYSIS, AND REPORT WRITING	~ $5000

A typical evaluation using purchased caged fish will therefore cost approximately $7000 and travel charges.

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APPENDIX I

OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY RESPONSES OBTAINED FROM 16 JUDGES IN THE EVALUATION OF YELLOW PERCH VARIABLY EXPOSED TO GUAIACOL

	Off-flavor Intensity Exposure Concentration (mg/L guaiacol)						Overall Acceptability Exposure Concentration (mg/L guaiacol)					
Judge	0 (hidden control)	0.01	0.10	0.50	1.00	2.00	0	0.01	0.10	0.50	1.00	2.00
A	4.9	2.9	2.2	5.5	4.5	3.5	2.0	3.8	4.1	1.5	2.7	3.2
B	1.5	1.1	4.7	3.3	2.4	5.4	5.4	6.0	1.3	3.2	2.4	1.0
C	1.2	1.0	0.7	5.6	1.7	5.0	3.8	4.2	5.7	1.2	3.1	1.5
D	0.8	4.7	2.9	3.8	5.1	5.8	5.8	2.7	4.4	3.4	2.8	1.5
E	2.7	3.9	3.3	1.9	2.3	4.6	1.3	2.8	2.0	4.7	5.2	3.3
F	1.4	2.4	0.6	6.1	3.4	4.9	5.3	3.8	6.5	0.9	2.8	2.0
G	2.1	1.2	3.8	5.2	4.5	1.7	4.4	5.6	1.8	1.0	2.4	5.0
H	0.8	1.0	0.9	0.7	0.7	0.8	5.1	4.9	4.8	4.7	5.0	4.9
I	4.5	0.8	3.0	2.1	4.0	2.4	2.4	4.7	3.0	4.5	2.7	3.4
J	3.2	1.8	3.4	4.7	4.5	2.8	3.0	5.3	2.9	1.7	1.9	3.8
K	3.4	3.0	4.9	2.1	2.7	5.2	3.6	4.0	1.9	5.4	4.7	1.0
L	2.3	3.7	1.1	6.5	6.2	5.9	4.3	4.0	4.6	0.8	1.0	0.7
M	3.7	1.2	0.6	4.1	5.8	5.3	2.6	4.7	5.6	2.3	0.9	0.9
N	4.3	3.3	2.6	5.1	2.0	1.3	2.5	3.3	3.7	1.9	4.4	5.2
O	4.5	1.5	1.0	1.2	5.0	4.7	4.2	4.9	5.5	5.2	3.4	4.6
P	3.0	4.1	4.9	1.3	2.0	1.6	2.1	1.3	1.8	5.0	4.0	4.6
Mean	2.77	2.35	2.54	3.70	3.55	3.81	3.61	4.12	3.72	2.96	3.09	2.91
Std. dev.	1.38	1.31	1.58	1.92	1.62	1.77	1.39	1.20	1.67	1.73	1.30	1.66
95% Confidence interval for mean	2.03 to 3.51	1.65 to 3.05	1.70 to 3.38	2.68 to 4.72	2.69 to 4.41	2.86 to 4.75	2.87 to 4.35	3.49 to 4.76	2.84 to 4.61	2.04 to 3.88	2.39 to 3.78	2.03 to 3.80

APPENDIX II

RANK SCORES AND STATISTICAL RESULTS OF OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY RESPONSES OBTAINED FROM 16 JUDGES IN THE EVALUATION OF YELLOW PERCH VARIABLY EXPOSED TO GUAIACOL

	Rank Score - Off-flavor Intensity Exposure Concentration (mg/L guaiacol)						Rank Score - Overall Acceptability Exposure Concentration (mg/L guaiacol)					
Judge	0	0.01	0.10	0.50	1.00	2.00	0	0.01	0.10	0.50	1.00	2.00
A	78.5	45.5	35.0	89.0	69.0	57.0	24.0	53.5	59.0	15.0	34.0	43.5
B	23.5	14.5	74.5	52.0	39.0	88.0	88.5	95.0	12.0	43.5	29.0	7.5
C	17.5	12.0	4.0	90.0	26.5	81.5	53.5	60.5	93.0	10.0	42.0	15.0
D	7.5	74.5	45.5	60.5	83.5	91.5	94.0	34.0	64.0	48.0	37.0	15.0
E	42.5	62.0	52.0	29.0	36.5	72.0	12.0	37.0	24.0	72.0	84.0	45.5
F	22.0	39.0	1.5	94.0	55.0	78.5	86.5	53.5	96.0	4.0	37.0	24.0
G	33.0	17.5	60.5	85.5	69.0	26.5	64.0	91.5	18.5	7.5	29.0	80.0
H	7.5	12.0	10.0	4.0	4.0	7.5	82.0	77.0	75.0	72.0	80.0	77.0
I	69.0	7.5	48.0	33.0	63.0	39.0	29.0	72.0	40.5	66.0	34.0	48.0
J	50.0	28.0	55.0	74.5	69.0	44.0	40.5	86.5	39.0	17.0	21.0	53.5
K	55.0	48.0	78.5	33.0	42.5	85.5	50.0	57.0	21.0	88.5	72.0	7.5
L	36.5	58.5	14.5	96.0	95.0	93.0	62.0	57.0	68.0	2.0	7.5	1.0
M	58.5	17.5	1.5	64.5	91.5	87.0	32.0	72.0	91.5	27.0	4.0	4.0
N	66.0	52.0	41.0	83.5	30.5	20.5	31.0	45.5	51.0	21.0	64.0	84.0
O	69.0	23.5	12.0	17.5	81.5	74.5	60.5	77.0	90.0	84.0	48.0	68.0
P	48.0	64.5	78.5	20.5	30.5	25.0	26.0	12.0	18.5	80.0	57.0	68.0
Σ Rank	684.0	576.5	612.0	926.5	886.0	971.0	835.5	981.0	861.0	657.5	679.5	641.5

Kruskal-Wallis adjusted H	11.918[a]	7.596
Critical value of the chi-square distribution at the 0.05 level	11.070	11.070
Nonparametric multiple comparisons by STP	Inconclusive	Not applicable

[a]Indicates significant differences in the data at $\alpha = 0.05$.

APPENDIX III

OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY RESPONSES OBTAINED FROM 15 JUDGES IN THE EVALUATION OF YELLOW PERCH EXPOSED AT SITES A, B, C, E, AND F

	Off-flavor Intensity					Overall Acceptability				
Judge	A	B	C	E	F	A	B	C	E	F
A	0.7	0.9	6.0	1.3	1.8	6.3	6.0	0.8	5.3	5.3
B	2.1	1.2	3.0	1.0	1.4	3.2	3.4	2.4	4.1	3.7
C	1.7	2.6	5.5	2.0	2.8	2.8	3.1	2.3	3.9	2.9
D	4.4	1.5	6.4	1.4	5.0	2.6	5.8	0.7	5.3	1.9
E	1.1	1.3	3.1	2.9	4.3	3.4	3.5	2.0	1.6	1.6
F	0.8	0.7	5.3	4.8	1.0	6.3	6.2	1.0	2.7	6.0
G	0.7	6.1	2.2	4.8	3.8	6.4	0.7	3.5	1.7	3.3
H	1.1	2.3	5.0	5.7	2.4	5.4	3.6	0.8	1.3	4.4
I	3.3	1.8	5.5	3.8	6.0	3.3	5.0	1.7	3.1	0.8
J	0.6	4.5	5.5	5.2	5.5	6.4	3.3	3.3	3.3	3.3
K	0.8	2.7	2.4	3.1	1.3	5.2	2.3	2.9	3.6	4.5
L	0.6	0.6	4.8	2.3	1.5	6.1	6.1	2.2	3.1	5.3
M	0.7	3.3	6.4	4.5	4.7	6.2	3.3	0.6	2.1	2.0
N	3.6	1.3	2.0	4.1	4.1	3.6	4.8	4.8	2.9	2.3
O	0.6	0.1	3.0	0.5	0.6	5.6	6.4	4.4	5.6	6.4
Mean	1.52	2.06	4.41	3.16	3.08	4.85	4.23	2.23	3.31	3.58
Std. dev.	1.26	1.60	1.60	1.68	1.79	1.50	1.68	1.35	1.36	1.70
95% confidence interval for mean	0.82 to 2.22	1.17 to 2.95	3.53 to 5.29	2.23 to 4.09	2.09 to 4.07	4.02 to 5.68	3.30 to 5.16	1.48 to 2.98	2.56 to 4.06	2.64 to 4.52

APPENDIX IV

OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY RESPONSES OBTAINED FROM 16 JUDGES IN THE EVALUATION OF WALLEYE TAKEN FROM SITES A, C, D, E, AND F (CANADIAN FISH INCLUDED IN ACCEPTABILITY TEST)

	Off-flavor Intensity					Overall Acceptability					
Judge	A	C	D	E	F	A	C	D	E	F	Canadian
A	1.2	5.3	5.8	5.8	6.3	5.9	1.0	1.4	0.8	0.7	6.0
B	0.6	5.0	6.3	5.8	5.3	6.2	1.5	0.6	0.9	1.8	6.2
C	2.0	1.6	2.0	2.0	2.4	3.2	3.0	3.1	2.9	3.0	2.7
D	2.8	5.3	3.2	5.2	5.1	2.9	1.7	3.6	1.0	2.3	4.5
E	1.5	1.2	4.1	2.0	0.6	2.0	2.5	0.8	1.9	3.6	3.0
F	1.3	5.9	0.8	4.8	1.2	5.6	2.6	6.2	2.9	5.9	5.5
G	0.6	1.5	3.0	1.2	2.5	6.5	5.4	4.6	5.8	4.8	3.6
H	0.7	1.6	0.6	4.4	1.1	6.0	4.9	6.2	2.2	5.6	0.8
I	1.1	4.6	4.1	6.0	2.1	5.0	1.5	2.3	0.9	4.1	5.6
J	1.0	6.1	4.6	5.7	4.6	5.3	1.5	3.4	2.3	3.5	6.4
K	1.8	4.1	0.8	3.6	3.7	4.4	2.4	5.4	4.0	3.3	4.9
L	0.7	2.6	0.8	1.3	2.5	5.6	2.3	5.2	2.2	2.0	3.9
M	5.3	5.2	4.7	6.1	1.0	1.8	1.9	2.1	1.0	5.3	5.7
N	4.7	1.4	4.8	5.4	6.1	1.2	4.5	3.6	2.3	0.9	6.0
O	5.4	5.4	0.6	0.9	0.7	1.4	1.8	6.3	5.3	5.3	6.2
P	0.6	2.4	2.2	5.2	3.3	6.5	4.3	4.2	1.5	3.3	4.9
Mean	1.96	3.70	3.02	4.09	3.03	4.34	2.68	3.69	2.37	3.46	4.74
Std. dev.	1.69	1.86	1.96	1.94	1.95	1.94	1.37	1.89	1.53	1.64	1.58
95% confidence interval for mean	1.06 to 2.86	2.71 to 4.69	1.98 to 4.06	3.06 to 5.12	1.99 to 4.07	3.31 to 5.37	1.95 to 3.41	2.68 to 4.70	1.55 to 3.19	2.59 to 4.33	3.90 to 5.58

APPENDIX V

RANK SCORES AND STATISTICAL RESULTS FOR OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY RESPONSES OBTAINED FROM 15 JUDGES IN THE EVALUATION OF YELLOW PERCH EXPOSED AT SITES A, B, C, E, AND F

	Off-flavor Intensity					Overall Acceptability				
Judge	A	B	C	E	F	A	B	C	E	F
A	9.5	14.0	71.5	21.5	29.5	70.5	64.5	5.0	57.5	57.5
B	33.0	19.0	43.5	15.5	24.5	31.0	39.5	21.0	48.0	46.0
C	28.0	39.0	67.5	31.5	41.0	24.0	29.5	19.0	47.0	26.0
D	55.0	26.5	74.5	24.5	62.5	22.0	63.0	2.5	57.5	13.0
E	17.5	21.5	45.5	42.0	54.0	39.5	41.5	14.5	9.5	9.5
F	12.5	9.5	65.0	60.0	15.5	70.5	68.5	7.0	23.0	64.5
G	9.5	73.0	34.0	60.0	50.5	73.5	2.5	41.5	11.5	35.0
H	17.5	35.5	62.5	70.0	37.5	60.0	44.0	5.0	8.0	49.5
I	47.5	29.5	67.5	50.5	71.5	35.0	54.0	11.5	29.0	5.0
J	5.0	56.5	67.5	64.0	67.5	73.5	35.0	35.0	35.0	35.0
K	12.5	40.0	37.5	45.5	21.5	55.0	19.0	26.0	44.0	51.0
L	5.0	5.0	60.0	35.5	26.5	66.5	66.5	17.0	29.0	57.5
M	9.5	47.5	74.5	56.5	58.0	68.5	35.0	1.0	16.0	14.5
N	49.0	21.5	31.5	52.5	52.5	44.0	52.5	52.5	26.0	19.0
O	5.0	1.0	43.5	2.0	5.0	61.5	73.5	49.5	61.5	73.5
Σ Rank	316.0	439.0	846.0	631.5	617.5	795.0	688.0	308.0	502.5	556.5
Critical X^2 at $\alpha = 0.05$			9.488					9.488		
Kruskal-Wallis adjusted H			23.03@					19.39@		
Multiple comparisons by STP*	A	B	F	E	C	A	B	F	E	C

@ Indicates significant differences in the data at $\alpha = 0.05$.
* Horizontal lines connect stations with similar means ($\alpha = 0.05$).

APPENDIX VI

RANK SCORES AND STATISTICAL RESULTS FOR OFF-FLAVOR INTENSITY AND OVERALL ACCEPTABILITY RESPONSES OBTAINED FROM 16 JUDGES IN THE EVALUATION OF WALLEYE TAKEN FROM SITES A, C, D, E, AND F (CANADIAN FISH INCLUDED IN ACCEPTABILITY TEST)

	Off-flavor Intensity					Overall Acceptability					
Judge	A	C	D	E	F	A	C	D	E	F	Canadian
A	19.5	64.5	79.5	72.0	79.5	83.5	10.0	13.5	4.0	2.0	86.0
B	3.5	58.0	72.0	72.0	64.5	90.0	16.5	1.0	7.0	21.0	90.0
C	31.5	27.5	31.5	31.5	36.5	46.0	43.0	45.0	40.0	43.0	38.0
D	41.0	64.5	43.0	61.0	59.0	40.0	19.0	52.5	10.0	32.0	61.5
E	25.5	19.5	48.0	31.5	3.5	25.5	36.0	4.0	23.5	52.5	43.0
F	22.5	74.0	11.0	56.5	19.5	78.5	37.0	90.0	40.0	83.5	76.0
G	3.5	25.5	42.0	19.5	38.5	95.5	74.5	63.0	82.0	64.0	52.5
H	8.0	27.5	3.5	50.0	16.5	86.0	66.0	90.0	28.5	78.5	4.0
I	16.5	52.0	48.0	75.0	34.0	68.0	16.5	32.0	7.0	57.0	78.5
J	14.5	77.0	52.0	70.0	52.0	71.5	16.5	49.0	32.0	50.0	94.0
K	29.0	48.0	11.0	45.0	46.0	60.0	35.0	74.5	56.0	47.5	66.0
L	8.0	36.5	35.0	61.0	44.0	78.5	32.0	69.0	28.5	25.5	55.0
M	64.5	61.0	54.5	77.0	14.5	21.0	23.5	27.0	10.0	71.5	81.0
N	54.5	24.0	56.5	68.0	77.0	12.0	61.5	52.5	32.0	7.0	86.0
O	68.0	68.0	3.5	13.0	8.0	13.5	21.0	93.0	71.5	71.5	90.0
P	3.5	40.0	11.0	22.5	38.5	95.5	59.0	58.0	16.5	47.5	66.0
Σ Rank	413.5	767.5	602.0	825.5	631.5	965.0	567.0	814.0	488.5	754.0	1067.5
Critical X^2 at $\alpha = 0.05$			9.488					11.070			
Kruskal-Wallis adjusted H			11.95@					20.08@			
Multiple comparisons by STP*	A	D	F	C	E	Canadian	A	D	F	C	E

@ Indicates significant differences in the data at $\alpha = 0.05$.
* Horizontal lines connect stations with similar means ($\alpha = 0.05$).

APPENDIX VII

FISH TAINTING ANALYSIS PROGRAM - OUTLINE

1. Specimen acquisition - specimens should, as much as possible, be of uniform size to minimize stimulus error resultant from size-related differences in flesh color, texture, and taste

   1.1 Electroshocking or netting of indigenous species at selected sites

   1.2 In situ receiving stream exposure (7-10 days) of fish in cages (Fig. 6) at selected sites

      1.2.1 Fish electroshocked or netted from uncontaminated natural sources, target species dependent on availability or suitability

      1.2.2 Fish obtained commercially from hatchery or other known high quality source, target species dependent on availability and suitability

   1.3 Exposure of specimens to potential tainting materials in laboratory test chambers - 96-hour exposure with semistatic (24 or 48 hours) solution replacement in aerated chambers of dechlorinated water. [Although yellow perch (Perca flavescens) are most available and generally applicable, some degree of target species flexibility may be accommodated relative to mill specific concerns]

2. Specimen processing - as soon as possible after collection or exposure, specimens should be killed, filleted (fillets should not be rinsed in collection or exposure water), double wrapped in aluminum foil, labeled, enclosed (one treatment each) in plastic bags, and frozen.

3. Evaluation of tainting of the fish flesh

   3.1 Analytical tool - sensory analysis panel consisting of 15 to 18 unbiased volunteers who do not object to the taste of fish. Panelists should exhibit

interest (motivation) and good health and should be regularly available for panel sessions. Panelists should be familiarized with test procedures; experience to be gained with participation.

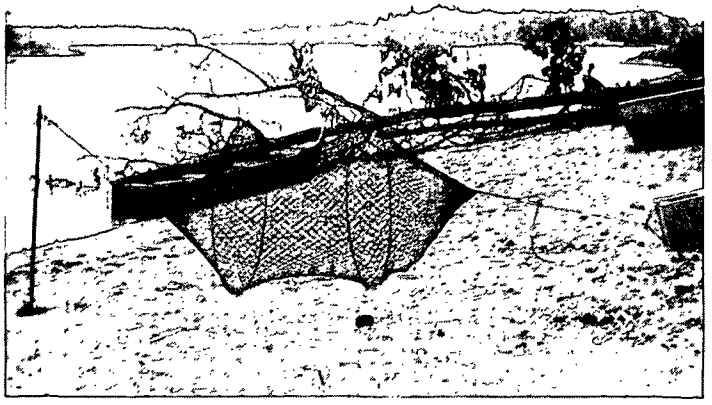

Figure 6. Holding net set used for caged fish exposure.

3.2 Test - unstructured linear scale scoring test; this continuous scale test is applicable to evaluations of sequences of sample sites or ranges of test concentration exposures, and minimizes differential word-connotation or number-preference biases.

3.3 Testing environment - testing area should be proximate to sample preparation area but free from cooking odors; other extraneous odors and outside distractions, e.g., smoking and cosmetic odors, noise, and visual distractions, should be avoided. Individual booths should be present or constructed, e.g., Fig. 7, to ensure independent judgments. Tests should be conducted during midweek and during midmorning or midafternoon hours.

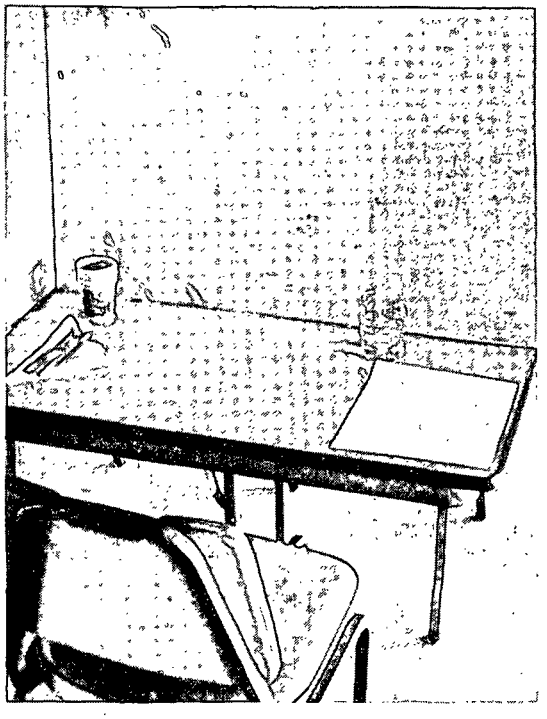

Figure 7. Preset sensory analysis booth.

3.4 Sample preparation

3.4.1 Remove fillets from freezer one day prior to scheduled evaluation and thaw overnight in a refrigerator.

3.4.2 Cut thawed fillets, similar portions of each, e.g., dorso-lateral muscle only, into pieces (≈ 1 x 1.5 inch).

3.4.3 Place samples into aluminum baking pans (one treatment per pan) without cooking oil or seasoning, and cover.

3.4.4 Bake in conventional oven at about 400°F (≈ 190°C) for 20 to 30 minutes.

3.4.5 Label appropriate number of covered glass Petri dishes: "C" for the reference control (off-flavor intensity test only); a

unique randomly determined three-digit code for each treatment and unidentified internal control.

3.4.6 After baking and transfer to labeled or coded dishes, samples should be organized, by treatment, and maintained on a food warming apparatus, e.g., under infrared lights (Fig. 8), before presentation to the panel.

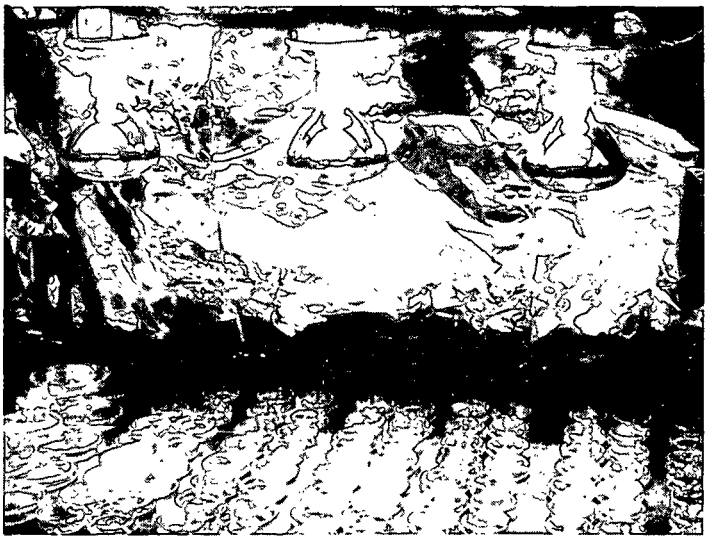

Figure 8. Infrared light setup for keeping samples warm prior to presentation to the panel.

3.5 Sensory testing - temporally separate panel sessions for off-flavor intensity and overall acceptability evaluations.

3.5.1 Preparation of the panel

3.5.1.1 Definition of parameters

3.5.1.1.1 Off-flavor intensity - the intensity of any detectable unusual or off-flavor compared with Sample "C"; Sample "C" is identified as a control sample to be tasted first and which is pre-

recorded for reference of no off-flavor near the left end of the off-flavor intensity scale.

3.5.1.1.2 Overall preference or acceptability - the acceptability of the sample as a food item.

3.5.1.2 Review of sensory evaluation procedures.

3.5.1.2.1 Single sample evaluation procedure

3.5.1.2.1.1 Masticate sample.

3.5.1.2.1.2 Void it into provided receptacle.

3.5.1.2.1.3 Record off-flavor intensity (relative to "C") or overall acceptability responses by making a vertical line across the line scale, i.e., on off-flavor intensity (Fig. 9) or overall acceptability (Fig. 10) ballots, at the point which best describes your assessment of the parameter.

3.5.1.2.1.4 Rinse mouth with water.

3.5.1.2.1.5 Wait about two minutes before proceeding to the next sample. Reference back or recalibration to Sample "C" is permitted at any time during off-flavor intensity evaluations.

3.5.1.2.2 Sequence of sample evaluations.

3.5.1.2.2.1 Proceed slowly and methodically.

NAME _____ DATE _____

I have been informed about the nature of the foods to be tasted by this panel

[ ]
(initial)

Directions

Taste sample _____ and make a vertical line across the horizontal line scale at the point that best describes your assessment of off-flavor intensity.

Off-Flavor intensity = intensity of any detectable unusual or off-flavor [Sample C (control) is recorded for reference]

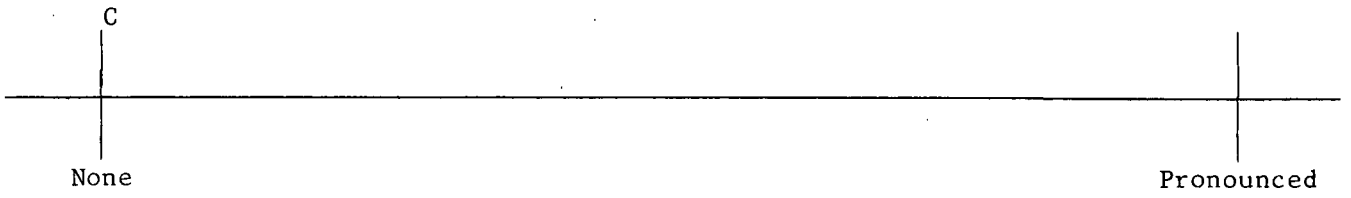

Figure 9. Off-flavor intensity sensory evaluation ballot.

NAME _____ DATE _____

I have been informed about the nature of the foods to be tasted by this panel

[ ]
(initial)

Directions

Taste sample _____ and make a vertical line across the horizontal line scale at the point that best describes your assessment of the acceptability of the sample as a food item.

Overall Preference (or Acceptability)

Figure 10. Overall preference or acceptability sensory evaluation ballot.

3.5.1.2.2.2 Evaluate samples in the order indicated by the sequence of coded ballots in the booths. (The evaluation sequence for coded samples is randomly predetermined for each judge to equalize contrast error; evaluation of only one sample per ballot better ensures independent judgments.)

3.5.2 Presentation of samples - a tray of uniformly warm samples ("C" and coded samples for off-flavor intensity tests, and coded samples only for acceptability tests) is presented to each panelist in preset (i.e., fork, napkin, rinse water, pencil, and appropriate ballots) individual booths for evaluation.

4. Data analysis

4.1 Numerical data acquisition - data obtained by superimposing a seven-part equal interval scale on the unstructured linear scale and recording appropriate numbers for responses [the off-flavor intensity scale is word- and "C" sample-anchored at 0.5 (no off-flavor) and word-anchored only at 6.5 (pronounced off-flavor). The overall preference or acceptability scale is word-anchored at 0.5 (dislike very much) and 6.5 (like very much)].

4.2 Statistical testing

4.2.1 Parametric methods - if homogeneity of variance and normality assumptions for parametric analysis of variance (ANOVA) testing are met, ANOVA and least significant difference tests can be applied.

4.2.2 Nonparametric methods - if above-mentioned assumptions are not met, the Kruskal-Wallis test and nonparametric multiple comparisons by simultaneous test procedure can be applied.